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ANTIBODY TO THE RNA-DEPENDENT DNA POLYMERASE OF HTLV-III: CHARACTERIZATION AND CLINICAL ASSOCIATIONS

ANNUAL REPORT

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We have found that the reverse transcriptase activity of the prototypic AIDS virus, HIV-1, was blocked in vitro by IgG derived from certain individuals infected with this retrovirus. A heterogeneous immune response for inhibition of enzyme function occurred. Catalytic activity was depressed by ≥ 50% with the use of 10ug of IgG from 11 of 16 HIV seropositive asymptomatic carriers, but from 0 of 8 seronegative controls and 2 of 12 patients with AIDS or ARC. It did not correlate with recognition of polymerase antigens by radioimmunoprecipitation. In a preliminary survey of serial serum samples it appears that loss of this inhibitor may be associated with development of clinical disease.

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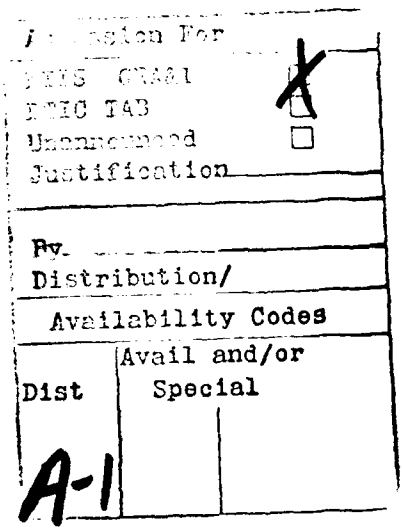
FOREWORD

The investigator(s) have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.



PROBLEM UNDER STUDY: The identification and characterization of anti-HIV reverse transcriptase antibodies in the sera of HIV-infected individuals, and correlation of their levels with clinical status.

BACKGROUND AND RATIONALE

The serum of many animals naturally infected with retroviruses contain antibodies which block the enzymatic activity of particulate reverse transcriptases (1-4). In some instances, reactivity occurs in the absence of detectable antibody to viral envelope or structural proteins (2). Correlations of antibody to reverse transcriptase (RT) with clinical status have been studied in cattle and cats. Bovine leukemia viral polymerase could be inhibited specifically by antibody from the serum of leukemic cattle, but not from the serum of infected animals experiencing solely a leukocytosis (2). In contrast, those cats that were exposed to feline leukemia virus (FeLV) and remained nonviremic or in a low state of viremia had antibody to FeLV polymerae (1). No such reactivity occurred in diseased animals.

Antibodies to the RT of human immunodeficiency virus type 1 (HIV-1), the prototypic etiologic agent of the acquired immune deficiency syndrome (AIDS), are readily detectable in the majority of HIV-seropositive individuals (5-7). These immunoglobulins, directed against proteins associated with integrase, RNase, and RNA-dependent DNA polymerase functions, can be recognized by immunoblotting and radioimmunoprecipitation techniques. They occur independently of the individual's clinical status (6,7). In addition, sera from asymptomatic West Africans infected with HIV-2, a closely related retrovirus, cross-react with HIV polymerase antigens (7). Such marked immunogenicity of a polymerase product has not been demonstrated for other mammalian retroviruses, including HTLV-I, in their respective hosts. In addition, some animals infected with retroviruses generate antibodies that bind to RT without neutralizing it (8). Investigation of the significance of these responses to HIV-1 formed the initial thrust of this contract.

EXPERIMENTAL METHODS

1. Patients Specimens. Serum samples were obtained from individuals with evidence of exposure to HIV as determined by immunoblotting for antigens in lysates of purified HIV. These samples were drawn from a serum bank initiated in 1982 (9), and containing single specimens as well as serial samples from individuals followed through 1987. The Walter Reed Staging Classification (10) for HIV infection was used to defined clinical stage.

2. Antibody Preparations. Immunoglobulin G (IgG) was isolated from each serum by ammonium sulfate fractionation and DEAE-cellulose column chromatography (11) or Zeta-Chrom 60 filter separation (CUNO Lab Products, Meriden, CT). F(ab')2 fragments of selected IgGs were prepared by digestion of the IgG with pepsin followed by S200 chromatographic purification (12).

3. HIv Stocks. H9, a CD4+ lymphoblastoiod T cell line permissive for the replication of HIV and partially resistant to its cytopathic and cytolytic effects, was infected with a strain of HIV-1 known as HIVB and used as a source for continuous production of particulate virus (13). These H9-HIVB cells were grown at a density of 1 x 106/ml in RPMI 1640 plus 10% FBS. Cell-free supernatants were collected and pooled at 4 d intervals, filtered through 0.45u membranes, and stored at-70oC.
Infectivity assays (13,18) indicated that 0.5ml of HIVB stock corresponded to a tissue culture infectious dose-50 (TCID50) of 1,000.

4. Neutralization of RT Activity. 0.75ml of clarified HIV stock was mixed with 0.25ml of a 30% solution of polyethylene glycol 3400 in water and precipitated at 4oC for 18 h in 1.5ml polypropylene tubes. Samples were then centrifuged at 10,000 g for 3 min. The pellet was resuspended in 25uL of virus-solubilizing buffer (0.8M NaCl, 0.5% Triton X-100, 0.5mM phenylmethylsulfonyl fluoride, 50mM Tris [pH 7.9], 1mM dithiothreitol, and 20% glycerol). IgG or IgG fragment was added to these assays for 4 h at 4oC.

5. Reverse Transcriptase Activity. Following exposure to IgG or control solutions, RNA template buffer was added. This consists of 64mM Tris (pH 7.9), 11mM MgCl2, 1.1mM dithiothreitol, 0.14mM dATP, 5 U of poly(rA).oligo(dT)12-18, and 5mCi of [3H-methyl]thymidine triphosphate (20 Ci/mmol sp. act.). The reaction is run for 1 h at 37oC with constant rocking, then stopped with 10% cold trichloroacetic acid (TCA) containing 0.1M sodium pyrophosphate, 1mM EDTA, and 10mM Tris (pH 7.9). Precipitates were collected on fiberglass filters

presoaked in 5% TCA, washed with 5% TCA, dried, and counted using liquid scintillant.

6. Metabolic Labeling and Radioimmunoprecipitation. HIV-infected H9 cells were harvested, washed in serum-free RPMI 1640, and resuspended at 5 x 106/ml in cysteine and methionine-free RPMI 1640 for 1 h at 37oC. 100uCi of [35S]cysteine and 100uCi of [35S]methionine (300 Ci/mmol) were added to each ml of cells, and the incubation continued for 4-6 h. A soluble cell lysate was prepared by disruption with lysate buffer (0.15 M NaCl, 0.05 M Tris [pH 7.2]. 1% Triton X-100, 1% sodium deocxycholate, and 0.1% SDS) and centrifuged at 10,000 g for 5 min. Lysates were cleared once with 10 uL of an HIOV seronegative control IgG (2mg/ml) bound to protein A-Sepharose beads (CL-4B; Sigma Chemical Co., St. Louis, MO). Portions were then reacted for 16 h at 4oC with 5 uL of IgG (1mg/ml) obtained from HIV seropositive individuals. Immunoprecipitates were eluted from the Sepharose beads in sample buffer containing 0.1 M dithiothreitol, 2% SDS, 0.08 M Tris (pH 6.8), 10% glycerol, and 0.2% bromphenol blue by boiling for 2 min. All samples were analyzed on a 7.5% acrylamide-resolving gel with a 3.5% acrylamide stacking gel in a discontinuous buffer system. Gels were impregnated with scintillator, dried, and radioactive bands detected by autoradiography.

7. Neutralization of HIV Infectivity. 1,000 TCID-50 of stock HIV were incubated with 1, 10 or 100 ug of IgG for 2 h at 25oC. Phytohemagglutinin-activated human peripheral blood mononuclear cells (2 x 106) were then added in RPMI 1640 plus 10% FBS plus 64 U/ml interleukin-2. Cultures were maintained for 18 h at 37oC, the medium was changed, and HIV replication assessed by RT determinations in the culture supernatants on days 7 and 14 after infection.

RESULTS

Note: The initial results have recently been published in:

Laurence J, A Saunders, J Kulkosky. 1987. Characterization and clinical association of antibody inhibitory to HIV reverse transcriptase activity. SCIENCE 235:1501-1504.

As shown in Fig. 1, incorporation of [3H]deoxythymidine monophosphate into DNA by HIV polymerase was blocked by IgG obtained from certain HIV-seropositive individuals. Using a screening dose of 10ug of whole IgG per reaction mixture, 11 of 16 asymptomatic HIV carriers inhibited RT activity by $\geq$ 50%. In contrast, 0 of the 8 samples from HIV seronegative controls and only 2 of 12 samples from patients with AIDS or AIDS-related complex (ARC) were suppressive at this concentration. The seronegative controls included 6 male laboratory workers, one female with HTLV-I-associated CD4+ lymphoma, and one pregnant female who gave a "false positive" reaction for antibody to disrupted HIV by enzyme-linked immunosorbent assay (ELISA). The seropositive groups were all homosexual males, age-matched to the control group. The symptomatic group included 6 AIDS patients (4 with Pneumocystis carinii pneumonia and 2 with Kaposi's sarcoma) and 6 ARC patients. Both individuals with anti-RT antibody activity >50% had Kaposi's sarcoma as their sole clinical manifestation of AIDS.

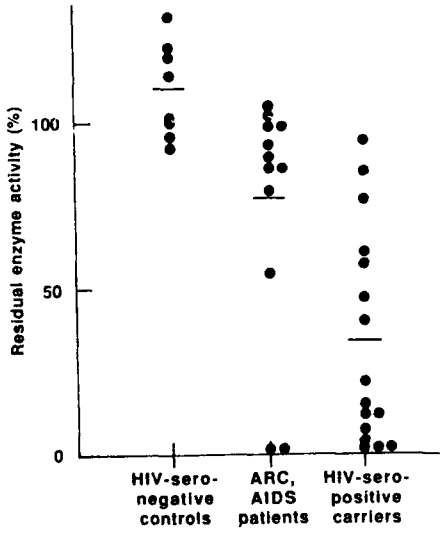


Fig. 1. Inhibition of HIV-associated RT activity by IgG. HIV virions, prepared from culture supernatants of $HIV_{HTLV\text{-}III_B}$-infected H9 lymphoblasts, were treated with virus-solubilizing buffer consisting of 0.8*M* NaCl, 0.5% Triton X-100, 1 m*M* dithiothreitol, 0.5 m*M* PMSF, 20% glycerol, and 50 m*M* tris (*p*H 7.9). Aliquots (25 µl) were incubated with 10 µg of total IgG, prepared from sera heated to 56°C for 30 minutes, and held at 4°C for 4 hours. Enzyme activity was then assayed. Template buffer (75 µl) consisting of 64 m*M* tris (*p*H 7.9), 11 m*M* $MgCl_2$, 1.1 m*M* dithiothreitol, 0.14 m*M* deoxyadenosine triphosphate, 7.5 mg/ml of poly(rA) · oligo$(dT)_{12\text{-}18}$, and 15 µ*M* [^{3}H]thymidine triphosphate were added to each virus sample. The reaction was run for 1 hour at 37°C with constant shaking and stopped with a solution of cold 10% trichloroacetic acid (TCA), 0.1*M* sodium pyrophosphate, and 2 mg/ml of yeast transfer RNA in 10 m*M* tris and 1 m*M* EDTA. Precipitates were collected on 26-mm fiber glass filters, washed with cold 5% TCA, dried, and counted. Enzyme activity of 100% represents [^{3}H]thymidine monophosphate incorporation in a reaction mixture containing phosphate-buffered saline (PBS) in place of IgG. IgGs obtained from HIV-seronegative asymptomatic controls, patients with AIDS, patients with ARC, and HIV-seropositive asymptomatic viral carriers were studied. Horizontal bars indicate mean residual RT activity for each group.

Dose-response curves for 3 representative HIV-seropositive carriers and 2 controls are illustrated in Fig. 2. The lack of cross-reaction with IgG from an individual with HTLV-I associated malignancy is apparent. A rabbit IgG prepared against disrupted HIV abrogated RT function (Fig. 2), while a rabbit antibody to the HIV transmembrane antigen gp41 had no effect.

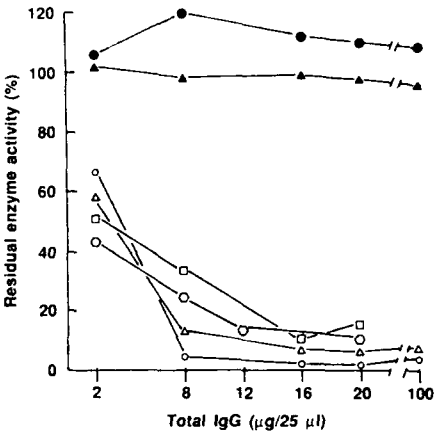


Fig. 2. Effect of increasing concentrations of total IgG prepared from heat-inactivated sera on HIV-associated RT activity. Assay conditions were as described in the legend to Fig. 1. Reaction mixture volumes were normalized with PBS. The closed symbols represent control IgGs, derived from an HIV-seronegative male (▲) and a female with HTLV-I–associated $CD4^+$ cell lymphoma (●). The open symbols represent IgG from HIV-seropositive asymptomatic carriers (○, △ and □) or a rabbit IgG to disrupted $HIV_{HTLV\text{-}III_B}$ virions (◯).

Five seropositive IgG samples that did not suppress RT activity at the 10ug screening dose were randomly chosen for further analysis. No specific inhibitory capacity was seen at concentrations up to 150 ug of IgG per reaction mixture. Some IgGs showed a slight and variable enhancement of HIV-associated RT activity (Figs. 1 and 2). This phenomenon has been demonstrated in other mammalian retroviral systems with normal sera (2), and attributed to protection of the enzyme from thermal inactivation during prolonged incubations.

Three time course for inhibition was evaluated by treatment of RT with IgG for various periods at 4oC and then adding RNA template. In the absence of inhibitor, RT Function decreased minimally upon extended incubation (Fig. 3). In contrast, the slope of the curve with an inhibitory IgG was steep, with >50% of the total suppression of catalytic activity occurring within 30 min. This experiment was repeated with the same concentration of F(ab')2 fragments of an inhibitory IgG. Similar results were obtained.

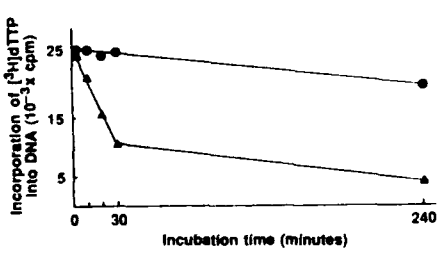


Fig. 3. Time course for inhibition of HIV RT activity. Solubilized virus was incubated with 10 μg of IgG from an HIV-seropositive asymptomatic carrier (▲) or with PBS (●) at 4°C for 10, 20, 30, or 240 minutes. Enzyme assays were performed, in duplicate, immediately after each incubation period, as described in Fig. 1.

The site of IgG action was examined by assaying for RT in the presence of increasing amounts of either solubilized virus or poly(rA).oligo(dT). Inhibition was diminished by raising the concentration of RT associated with disrupted HIV in the final reaction mixture, whereas altering the template concentration had no effect (Fig. 4). These observations, coupled with the requirement for incubation of the polymerase with IgG for functional inactivation, further suggest enzyme binding as the most likely mechanism of action.

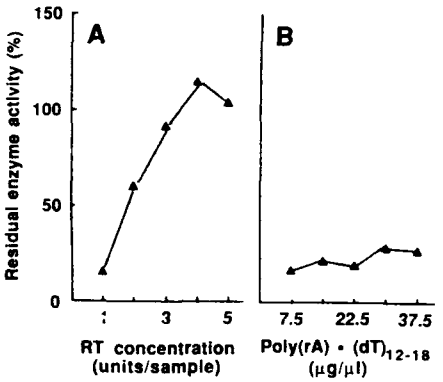


Fig. 4. Effect of increasing the concentration of disrupted HIV (**A**) or its template (**B**) on the inhibition by total IgG of reverse transcription of poly(rA) · oligo(dT). Solubilized HIV virions were incubated with 10 μg of IgG from an HIV-seropositive asymptomatic carrier at 4°C for 4 hours and then assayed as outlined in Fig. 1. The only variations were the final concentrations of disrupted virus (A) or template (B) in the reaction mixtures. One unit of RT activity corresponds to solubilization of approximately 1×10^3 tissue culture infectious doses of HIV.

Correlation of anti-RT activity with clinical outcome was approached by a retrospective survey of the 16 asymptomatic HIV-seropositive individuals presented in Fig. 1. All have been followed for a mean of 3 years, with a range of 2-5 years. At initiation of sample collection these persons corresponded to WR stage 0 (12 of 16) or WR stage 3 (4 of 16, no lymphadenopathy). Of the 11 with anti-RT activity (≥ 50% inhibition at 10ug of IgG), 6 have maintained these titers and 5 have lostthis capacity. All of 6 with persistent inhibitors remain asymptomatic. All of 4 who lost this activity developed AIDS or ARC within 6-12 months after recognition of decreasing titer. Of the remaining 5 individuals lacking RT inhibitors, 2 have developed ARC, 2 remain clinically stable, and one was lost to follow-up.

To further characterize the proteins recognized by our inhibitory IgGs, we analyzed immunoprecipitates of [35S]cysteine and [35S]methionine-labelled proteins from H9-HIVB lymphoblasts by SDS-PAGE. A representative sample of 9 IgGs from HIV seropositive subjects, including serial specimens ≥ 2 years apart obtained from 3 individuals, were tested. As illustrated by 8 selected samples in Fig. 5, these reactions occurred independently of the ability of the immunoglobulin to block HIV reverse transcriptase function. In agreement with another larger survey (14), there was also no difference in pattern of antibody recognition of HIV-related envelope (gp160 and gp120) or structural (p55, p24, and p17) antigens in symptomatic versus asymptomatic viral carriers (Fig. 5).

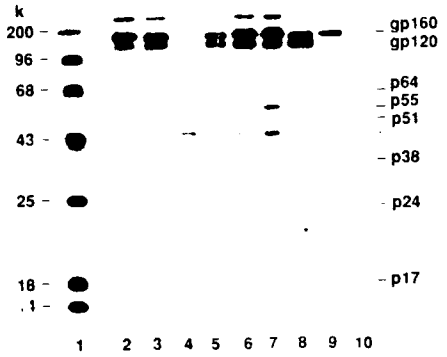


Fig. 5. Radioimmunoprecipitation of HIV proteins by selected IgGs. Lysates were prepared from $HIV_{HTLV\text{-}III_B}$-infected H9 lymphoblasts labeled with [^{35}S]methionine and [^{35}S]cysteine as previously detailed (*17*). They were precipitated with total IgG derived from sera of the individuals shown below.

Lane	Patient	Year serum was obtained	Diagnosis	IgG RT inhibitor
1	Molecular weight markers			
2	Bah	1983	Asymptomatic carrier	+
3	Bah	1985	AIDS	−
4	El	1983	Asymptomatic carrier	+
5	El	1985	AIDS	−
6	Dou	1983	AIDS	−
7	Gre	1983	Asymptomatic carrier	+
8	Bir	1985	ARC	−
9	Cha	1985	ARC	+
10	Control IgG from an HIV-seronegative individual			−

The possibility that these anti-RT IgGs correlated with existence of other antibodies with reactivity against neutralizing envelope epitopes was next investigated. This latter area is controversial. Virus neutralizing factors have been found in >50% of serum samples obtained from AIDS and ARC patients (15), and in a higher number of HIV seropositive asymptomatic carriers (16). Clinical correlations in patients followed over time has been weak, however. The assays typically involve single HIV isolates as standard targets, albeit envelope variability is well documented among HIV strains, even from a single individual (17). We attempted to link HIV neutralization in vitro with anti-RT activity, using IgG from 10 of the asymptomatic carriers. Using $\geq$ 75% inhibition of enzyme activity as the criterion for neutralization, we found most samples effective at 10-100 ug IgG, without regard to the patient's clinical status or the anti-RT capacity of the IgG.

CONCLUSION AND DISCUSSION

We have found that the reverse transcriptase activity of the prototypic AIDS virus, HIV-1, was blocked in vitro by IgG derived from certain individuals infected with this retrovirus. A heterogeneous immune response for inhibition of enzyme function occurred. Catalytic activity was depressed by $\geq$ 50% with the use of 10ug of IgG from 11 of 16 HIV seropositive asymptomatic carriers, but from 0 of 8 seronegative controls and 2 of 12 patients with AIDS or ARC. It did not correlate with recognition of polymerase antigens by radioimmunoprecipitation. In a preliminary survey of serial serum samples it appears that loss of this inhibitor may be associated with development of clinical disease.

The origin of these inhibitory antibodies is unclear. They presumably arise from immunizations against enzyme from circulating disrupted virions or enzyme present on the infected cell membrane either as a mature protein or in the form of a group antigen-polymerase (gag-pol) polypeptide precursor. Catalytically inert gag-pol protein has been found on the surface of cells infected with mutants of murine leukemia virus blocked at late stages of virion assembly (19). RT activity in complexes without viral structure has also been observed in bone marrow cells transformed by reticuloendotheliosis virus (20). Polymerase-IgG binding without inactivation of the catalytic activity of the RT, typical of the AIDS and ARC patients here examined, may have several explanations. IgGs recognizing divergent epitopes may be responsible for immunoprecipitation of pol gene products seen with samples from early asymptomatic as opposed to late symptomatic HIV infected persons. For example, antibodies to peptides predicted from the 5' end of mammalian retroviral pol genes may inhibit RT activity whereas antibodies to peptides from the midportion of the gene do not (21).

The possibility that antibodies to non-envelope structures are effective in neutralizing HIV in vitro has been raised (22). Antibody to RT similarly might interfere with virion assembly or budding. Whether such a mechanism is responsible for the clinical correlations observed here is unknown. Alternatively, IgGs with anti-RT capacity are surrogate markers for protective cellular immune responses or for other antibodies with reactivity against neutralizing envelope epitopes.

CONFIRMATORY DATA FOR THE CLINICAL CORRELATIONS OBSERVED IN OUR PILOT STUDY

In our recent publication of some of the above findings (23) we concluded that: "If these data re reproduced in larger

surveys of HIV-infected individuals, the assay could serve as a marker for disease progression, similar to the correlation of anti-RT activity with clinical status observed in other mammalian retroviral models." Two recent papers (24,25), one quoting our work, have now replicated our data with anti-RT IgGs, and one recent review on vaccine strategies in AIDS (26) has used our model for predicting that alternate targets for HIV vaccines, apart from gp120 and gp160, are possible.

In one of these studies (24), involving 33 patients, an inverse correlation was found between the presence of anti-RT antibodies and the ability to isolate HIV from peripheral blood lymphocytes of those individuals (Table 1). Increased frequency of HIV isolation and the detectability of circulating core p24 antigen correlates with declining clinical status (27).

Table 1. Association between anti-RT antibody and HIV isolation*

Sample	Number	No. HIV Seropositive	No. with anti-RT Activity	No. (%) with Isolatable HIV
Homosexual male	33	33	33	6 (18%)
	16	16	0	10 (63%)
	33	0	0	0
Blood donors	4	0	0	0

*Data taken from reference 24.

In the other study (25), 9 asymptomatic viral carriers, 10 lymphadenopathy patients, and 8 ARC patients were examined for IgGs inhibitory to HIV RT. The mean quantity of IgG required to inhibit RT activity by $\geq$ 50% was 7.6 ug for the asymptomatics, but 20.0 ug for individuals with the lymphadenopathy syndrome and 30.5 ug for ARC patients.

FUTURE GOALS

In the next 2 years of this contract we have five objectives.

1. Screen a much larger number of individuals for which serial serum specimens are available, to firmly establish the clinical correlation of absence of anti-RT activity with subsequent development of clinical disease.

Toward this goal we have obtained 120 samples, representing 15 individuals at various times from asymptomatic carrier through clinical AIDS, from Dr. Zvi Bentwich, Director of Clinical Immunology, Ther Kaplan Hospital, Rehovot, Israel. We also hope to obtain additional samples from the U.S. Army.

2. Correlate anti-RT activity with another possible "prognosticator" in HIV infection, the p24 antigen capture system. We have this system, purchased from Abbott Laboratories, working in our lab. We will also attempt to correlate this activity with CD4+ T helper cell function.

3. We have established a collaboration with Dr. Susan Zolla-Pazner of New York University. She has isolated a series of human B cell hybridomas and Epstein-Barr virus transformed human B cell lines which secrete antibodies against various HIV-1 epitopes. She has recently given us 2 IgM anti-RT reagents, which recognize HIV-RT protein by western blotting. We will evaluate these reagents for activity against RT catalytic activity.

4. We recently received an isolate of HIV-2 from Dr. P. Kanki of the Harvard School of Public Health. We wish to determine whether our anti-HIV-1 RT antibodies will recognize the catalytric activity of HIV-2. Similarly, if HIV-2 sera were available, we could evaluate the prognostic significance of our assay in these individuals.

5. As a long range goal we plan to epitope map HIV RT using synthetic peptides prepared from regions of the polymerase conserved among mammalian RTs.

REFERENCES

1. P.C. Jacquemin, C. Saxinger, R.C. Gallo, W.D. Hardy, M. Essex, Virology 91, 472(1978).

2. K.D. Wuu, D.C. Graves, J.F. Ferrer, Cancer Res. 37, 1438(1977).

3. S.A. Aaronson, W.P. Parks, E.M. Scolnick, G.J. Todaro, Proc. Natl. Acad. Sci. 68, 920(1971).

4. G. Bauer and H.M. Temin, J. Virol. 34, 168(1980).

5. K.S, Steimer et al., J. Virol. 58, 9(1986).

6. F. di Marzo Veronese et al., Science 231, 1289 (1986).

7. J.S. Allan et al., Blood 69, 331 (1987).

8. M.R. Robert-Guroff, A.W. Schrecker, B.J. Brinkman, R.C. Gallo, Biochemistry 16, 2866(1977).

9. J. Laurence et al., N. Engl. J. Med. 2 , 1286(1984).

10. R.R. Redfield, D.C. Wright, E.C. Tramont, N.Engl. J. Med. 314, 131(1986).

11. J.L. Fahey and A.P. Horbett, J. Biol. Chem. 234, 2645(1959).

12. M. Popovic, M.G. Sarngadharan, E. Read, R.C. Gallo, Science 224, 497(1984).

13. J. Laurence, J. Kulkosky, S.M. Friedman, D.N. Posnett, P.O.P. Ts'o, J. Clin. Invest. 80, 1631(1987).

14. J. E. Groopman et al., J. Infect. Dis. 153, 736(1986).

15. S. Rasheed et al., Virology 150, 1(1986).

16. M. Robert-Guroff, M. Brown, R.C. Gallo, Nature 316, 72(1985).

17. B.H. Hahn et al., Science 232, 1548(1986).

18. J.S. McDougal et al., J. Immunol. Methods 76, 171(1985).

19. O.N. Witte and D. Baltimore, J. Virol. 28, 750(1978).

20. T.C. Wong and C.Y. Kang, J. Virol. 28, 34(1978).

21. R.A. Lerner, Nature 299, 592(1982).

22. P.S. Sarin, D.K. Sun, A.H. Thornton, P.H. Naylor, A.L. Goldstein, Science 232, 1135(1986).

23. J. Laurence, A. Saunders, J. Kulkosky, Science 235, 1501(1987).

24. K. Sano et al., J. Clin. Micro. 25:2415, 1987.

25. R. Chatterjee, C.R. Rinaldo Jr., P. Gupta, J. Clin. Immunol. 7, 218(1987).

26. J. Salk, Nature 327, 473(1987).

27. F. de Wolf et al., Brit. Med. J. 295, 569(1987).

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